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In vitro testing protocols for the cruciate ligaments and ligament reconstructions

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Abstract The techniques that have been used to characterize the biomechanical behavior of the knee, cruciate ligaments, and cruciate ligament replacements differ, making comparisons between studies difficult or, at times, impossible. Therefore, it is important to standardize the testing protocols and techniques that describe the biomechanical behavior of the knee and cruciate ligaments. This will allow investigators to express opinions with respect to the interpretation of data, rather than based on differences between testing techniques. Standardized techniques are proposed to locate the origins of the tibial and femoral coordinate systems, and thus, allow comparisons of knee kinematics (e.g., displacements and rotations) between investigations. Standard techniques that can be used to measure the load-displacement behavior of the knee are described, and important considerations that should be appreciated with respect to preparing and testing of the joint are summarized. It is important to evaluate the single cycle load-to-failure characteristics and the cyclic loading response of an anterior cruciate ligament graft, and techniques to evaluate cruciate ligament graft fixation are proposed. The strengths of different models to characterize the biomechanical behavior of the knee are reviewed.

Key words Knee · Biomechanics · Anterior cruciate ligament · Graft fixation

Introduction

The knee joint is the largest and most complex joint in the human body. The ligaments and joint capsule which provide structural stability to the knee are particularly vulnerable to injury due to the large moments that can be created through the forces acting on the long lever arms of the lower limb. Therefore, it is not surprising that the knee is one of the most frequently injured joints [18]. An injury to the knee, such as disruption of the anterior cruciate ligament (ACL), may result in disability, because this injury alters the normal knee kinematics and stability, and therefore locomotion. One of the challenges that clinicians and researchers face is establishing mutually agreed upon techniques to evaluate knee and ligament biomechanics. There is much confusion in the literature caused by different techniques to apply load to a knee/ligament graft and measure the biomechanical response. The objective of this paper is to propose standard techniques to measure knee and ligament biomechanics.

Description of coordinate systems and knee motion

To the untrained observer, the knee joint may appear to function as a simple ‘pinned’ hinge (ginglymus) with flexion-extension rotation the only apparent motion between the femur and tibia. However, the motion characteristics of the knee joint are complex, requiring a full six degrees-of-freedom (three translations and three rotations) to completely describe the coupled, or simultaneous, joint motions. An example of coupled motion is demonstrated with flexion rotation of the knee from the extended position. With this rotation, there is a coupled posterior movement of the femoral contact regions of the tibial surface in the sagittal plane and an internal rotation of the tibia relative to the femur in the tibial transverse plane. Using the Eulerian-based coordinate system described by Hefzy and Grood, the translations and rotations can be described in anatomically referenced directions [12]. Although many different types of coordinate systems have been used to describe three-dimensional (3D) knee motion, this system is appealing because it allows joint rotation to be expressed in terms familiar to clinicians. For example, Grood and Noyes have applied the 3D coordinate system to the interpretation of various clinical examination techniques and have developed a 'bumper model' of the knee joint [10]. This model is useful for describing the soft-tissue restraints to anterior-posterior (A-P) translation and internal-external rotation of the knee joint. In addition, the model can be applied to demonstrate the types of tibiofemoral subluxations that are produced when different soft-tissue structures are disrupted. Application of this approach may aid in the examination of injuries to the knee ligaments and capsular structures. In addition, it will allow effective communication between clinicians and researchers.

The location of the origins of the tibial and femoral coordinate systems will influence the apparent magnitudes of displacement between these systems as the knee is moved. Therefore, it is important to describe and standardize the locations of the origins of the femoral and tibial coordinate systems in relation to each bone. The locations of these coordinate systems are the natural extension of our earlier report [4] which defined the location of the coordinate system such that it could be digitized directly from bone and visualized on radiographs. The approach used for the femur was to use scaling dimensions appropriate to individual knees. Thus, the diameter of the posterolateral femoral condyle, which is recognized as being spherical, should be used as the unit of measurement on radiographs, with a measurement system that is based at the center of this circle. A proximal-distal line was referenced to the posterior cortex of the femur. The femur is curved in the sagittal plane, and therefore standard positions are required to define the measurement line for accurate positioning of this axis. To establish the femoral coordinate system, a straight line should be drawn through two points on the posterior outline of the femur, one and two posterolateral femoral condyle diameters proximal to the over-the-top position; this will be referred to as the proximal-distal axis. It is important to recognize that knees vary greatly in size, and therefore, it is not appropriate to define the measurement positions as absolute units of measure, such as millimeters; the condyle diameter method will give measurement positions that are appropriately scaled. A medial-lateral axis should be constructed such that it runs parallel to the centers of the posterior aspects of the femoral condyles (medial and lateral) and intersects with the proximal-distal axis. This intersection should produce orthogonal alignment in the coronal plane. A third axis directed AP should be constructed orthogonal to the medial-lateral and proximal-distal lines. Mathematically, this can be determined by taking the vector cross-product of the proximal-distal and mediolateral axes. For the tibial coordinate system, the posterior border of the tibial shaft is used as a reference. Again, standard positions are identified to describe the proximal-distal line. A proximal-distal axis is placed through points one and two femoral condyle diameters distal to the tibial plateau and aligned parallel with the posterior cortex of the tibia. The posterior aspect of the tibia is usually straight in this region and easy to identify on an X-ray. A mediolaterally directed axis can be identified by constructing a line tangent to the lateral tibial plateau in the coronal plane and intersecting the proximal-distal cortex line with an orthogonal alignment. In normal knees, this intersection will typically occur between the tibial eminences. An AP axis is then constructed perpendicular to the medial-lateral and proximal-distal axes and aligned orthogonally. Again, this can be determined by calculation of the vector cross-product.

Appropriate models to study knee biomechanics

There are many different models that can be used to study knee joint biomechanics. The choice of an appropriate one depends upon the research hypothesis that is to be tested, and therefore, it is not appropriate to recommend a specific model. However, several observations can be made. The 'knee', or stifle, joints of very many species have several similarities to the human knee – the basic design was well established long before the dinosaurs evolved – but the caprine, canine, ovine, and porcine models have been most popular for in vivo simulations. All have similar primary ligamentous complexes (anterior and posterior cruciate ligaments, ACL, PCL, medial and lateral collateral ligaments, MCL, LCL), menisci, and bicondylar joint geometry. However, most quadrupeds have a knee that does not extend beyond 30°. There has been equal interest in the use of canine, caprine, and ovine models for studying knee ligament reconstruction. These animals are considered appropriate for studying most healing responses. Additionally, the anatomy and biomechanics of the ovine and caprine stifle joints have been characterized with reference to their use as models of the human knee [11, 17]. The rabbit and primate models have been used to a lesser extent to study ligament biomechanics and reconstructions [5, 7, 14].

For work in vitro, human knees are the appropriate choice if they are within a certain age range and free of specific disease. All investigations should report the age, gender, cause of death, and complicating illness associated with death. For example, studying fixation of ACL grafts with osteoporotic bone will provide results that differ greatly from those from young bone. At times, these results may be highly variable. We recommend testing male specimens that are 65 years old or less, and female specimens that are 50 years old or less. These specimens should be free of metabolic disease such as that associated with diabetes.

It is usually impractical to perform mechanical testing on a joint at the time of acquisition, although Viidik and Sandquist [23] showed that storage for 48 h post-mortem had no significant effect on the cruciate ligaments. Therefore, specimens should be acquired and stored appropriately. Specimens should be wrapped in saline-soaked gauze, double bagged in sealed polyethylene bags, and stored at –20°C or below until testing can be performed. Woo and colleagues have demonstrated that storage of rabbit knee specimens at –20°C for up to 3 months did not alter the mechanical or structural properties of the MCL [25]. Storage for time intervals longer than this may produce changes, but little information is available regarding this issue at the moment.

On the day of mechanical testing, limbs should be removed from the freezer and thawed at room temperature. Specimens should not be immersed in tap water because it is not balanced with normal physiological fluids. The joints should undergo careful dissection to remove the dermal layer and surrounding tissues. This procedure can include removal of the periosteum from the tibial and femoral diaphyses. Care should be taken not to violate the joint capsule during dissection; this will expose the primary knee ligaments. If an isolated knee specimen is used, it is advisable to secure the fibula to the tibia via cortical bone screws, to re-establish the normal stability of the interosseous membrane, since this affects the LCL. Saline-soaked gauze should be used to keep the soft-tissue structures moist at all times during specimen preparation and potting. Care should be taken not to repeatedly spray specimens with saline because evaporation of the water will leave an abnormally high concentration of salt on the joint; this may produce an imbalance in the hydration of the tissues during specimen preparation, potting, and testing. The potting procedure should be designed to ensure a reproducible location of the tibial and femoral axes of rotation as well as symmetry of loading for the right and left limbs. One approach is to locate the long axis of the posterior portion of the tibia collinear with the long axis of a pipe, and align the center of the tibial plateau with the center of the pipe. This may require a stab incision through the patellar tendon, to allow a probe to be located between the tibial spines [3]. A similar alignment procedure can be used for the femur. To minimize the unsupported length of the tibia and femur, each pipe should be positioned as close as possible to the joint line while preventing contact with the joint capsule. Each limb can be secured in place within the pipes by pouring casting material, such as polyester resin with fiberglass reinforcement, PMMA bone cement, Wood's metal, or plaster of Paris, between the bone and metal pipe. To enhance the purchase of the casting material to bone, it is useful to apply a series of K-wires or screws into the diaphyseal regions of the femur and tibia and to file grooves in their surfaces. This will allow a rigid purchase of the bone via the casting material. Careful attention should be given to the use of casting materials that are exothermic, which could heat and dry out a specimen during preparation. After the casting material solidifies, the joint biomechanics can be evaluated.

Measurement of the load-displacement response of the knee

Experimental studies of the intact knee joint can use either a flexibility or a stiffness approach.

The flexibility approach involves observing or measuring a displacement produced by an applied joint load; a ligament is cut, and the procedure is repeated. The relative difference in displacement is then used to establish the importance of that ligament. This method is analogous to clinical laxity examination (i.e., the Lachman test), in which the clinician applies a load and estimates the resulting joint displacement. This technique is useful for evaluating the sensitivity of knee laxity tests to injuries created in human cadavers. One limitation of this technique is that the difference between the behavior of a knee joint before and after excision of the ligament does not necessarily indicate that cutting of the ligament was wholly responsible – its loss may affect other interacting structures, and the results are cutting sequence dependent.

The stiffness approach establishes the role of a particular ligament by applying a predetermined displacement while simultaneously measuring the load applied to the knee joint, cutting the structure under investigation, and repeating the displacement while documenting the decrease in load that results. This methodology is useful for determining which motions are resisted by each structure and the relative importance of the structure. It can be applied to discern the functions of each ligament (e.g. Butler et al. [9] showed that the ACL is the primary restraint to tibial anterior drawer) or even to parts of ligaments (e.g. Amis and Dawkins [2] showed how different fiber bundles of the ACL act when the knee is tested while flexed or extended).

A comparison between the flexibility and stiffness methodologies reveals that the latter produces results independent of the order in which the ligaments are sectioned and therefore, is a more direct approach. The results from the flexibility approach are governed by the order of ligament cutting, and thus, their outcomes may be difficult to interpret.

Techniques to evaluate A-P laxity of the knee

One of the most common techniques to evaluate the biomechanical behavior of the knee is to apply A-P load and

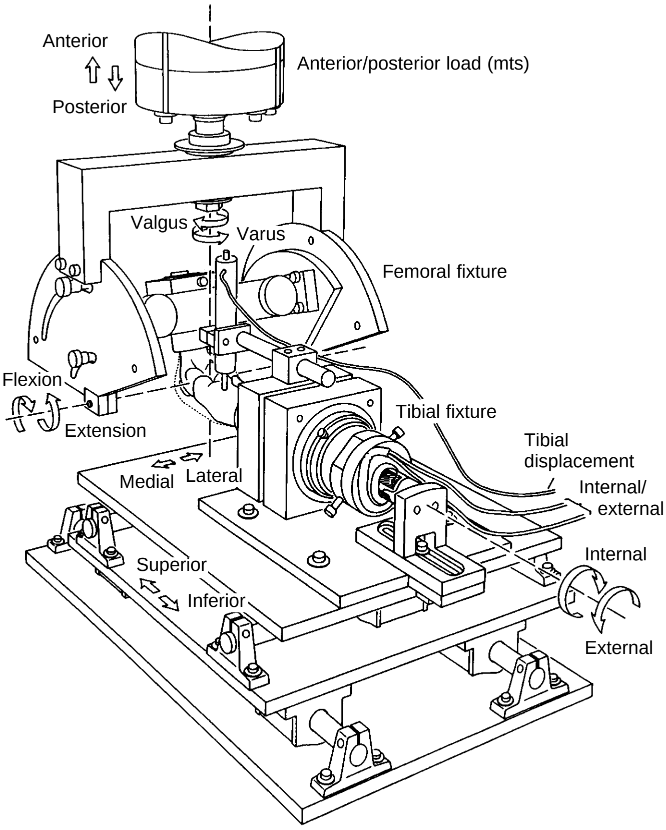

Fig. 1 The anterior-posterior (A-P) knee laxity fixture was designed to accommodate positioning and adjustment of the knee with a full six degrees-of-freedom. It has pins to align the rotational axes of the fixture with the anatomically based rotational axes of the knee (*dashed lines*). Load is applied to the femur through the materials test system while the tibia is held in the horizontal plane (displacements in the medial-lateral and superior-inferior planes of the tibia are allowed at all times). With reference to the anatomic planes of motion, the degrees of freedom of the laxity fixture were AP, medial-lateral, and superior-inferior displacements, along with flexion-extension, varus-valgus, and internal-external tibiofemoral rotation. The flexion-extension and internal-external tibiofemoral rotations were locked while A-P loads were applied across the tibiofemoral joint and unlocked for adjustment of the knee flexion angle. (Reproduced with permission from [6])

measure the resulting A-P displacement. The A-P laxity fixture should accommodate positioning and adjustment of the knee with a full six degrees-of-freedom [6] (Fig. 1). In addition, the degrees-of-freedom of the laxity fixture should be referenced to the anatomic planes of knee motion: A-P, medial-lateral, and superior-inferior displacement, flexion-extension, varus-valgus, and internal-external rotation. The laxity fixture should be designed with alignment pins that permit the axes of rotation of the loading fixture to be aligned with the anatomically based rotation axes of the knee. If this is not done, coupled secondary motions could result. For example, if the longitudinal rotation axis of the tibia is offset laterally in its mounting, an anterior drawer force applied to the mounting will also cause an external rotation. One approach to loading the knee is to use the laxity fixtures such as those designed by Sullivan et al. [20], Beynnon et al. [6], and Amis and Scammell [3] (Fig. 1). These devices are used as fixtures in a materials testing machine. When A-P laxity is evaluated, these fixtures can have a variable number of degrees of freedom. Earlier work on the knee used single degree-of-freedom attachments which constrained all secondary motions: only A-P displacement of the tibia was allowed at the chosen angle of knee flexion [1]. It is most common now for A-P laxity to be tested in a five degrees-of-freedom fixture, in which A-P displacements are applied at a fixed angle of flexion and the tibia is free to translate in the proximal-distal and medial-lateral directions and to rotate in the varus-valgus and internal-external directions. The principal advantage of the greater complexity of the latter arrangement is that forces are not ‘locked in’ by restraining secondary motions. One example of this could be tibio-femoral impingement, due to an A-P slope of the tibial plateau. This fixture can be used to apply A-P loads and measure the resulting displacements; varus-valgus torque and measure the resulting rotations; and internal-external torque and measure the resulting rotations. These measurements are quite useful when described in conjunction with the anatomical coordinate system previously described.

It is important to appreciate the coupled rotations and translations that occur at the knee during adjustment of the knee flexion angle in a test rig. During adjustment, internal-external rotation of the tibia as well as varus-valgus angulation should remain unlocked and free to rotate. At the desired flexion angle, the tibia should then be rotated internally and externally by the investigator and the midpoint of rotation established. The A-P load-displacement response of the knee is dependent on whether internal-external rotation of the tibia relative to the femur is locked or unlocked [3], and therefore, this must be specified in this neutral position, or some other position of rotation.

It is important to consider the number of load cycles that are used to evaluate the biomechanical behavior of the knee. For example, A-P laxity should be evaluated when the behavior of the knee has stabilized after several complete A-P loading cycles. The first load cycle is of interest and is part of preconditioning the specimen. Later cycles should demonstrate reproducible load-displacement behavior for normal knee specimens. In some situations, such as studying cruciate ligament graft fixation, it may be interesting to measure how the load-displacement behavior of the knee changes with multiple loading cycles applied to the knee, and therefore all loading cycles from the first through the last may be examined.

It is also important to define the load magnitudes that are applied to the knee joint. A-P knee laxity should be defined as the A-P displacement of the tibia relative to the femur that occurs between limits of 150 N (anterior) and –150 N (posterior) shear loads for studies of the ACL

(ACL graft). This convention is chosen to load the PCL and use it as a reference. If the PCL is not involved with surgery, it is reasonable that the reference for displacement measurement is a posteriorly applied load to the tibia, which engages the PCL. The same could be said of the ACL in studies of the PCL or PCL grafts. Tests should be performed using loading rates that are approximately 10 s per A-P loading cycle. This will allow an evaluation of the knee in a controlled manner that does not include inertial loadings.

A-P laxity evaluation should be performed at a selection of knee flexion angles. It is usually important to study the knee near extension (20°), mid-flexion (60°), and at 90° of flexion. This is important because of the multi-bundle nature of the cruciate ligaments and the resulting flexion angle dependence of knee joint laxity. As well as laxity at the extremes of displacing force, it can also be useful to examine stiffness, since ACL integrity is associated with a 'hard end-point'. This is characterized as the slope of the relationship between load applied to the tibiofemoral joint versus its displacement, and is expressed in terms of N/mm at a chosen force such as 50 and 100 N [13]. The inverse of this value is known as compliance.

The same approaches that we used to characterize A-P knee laxity can be followed to evaluate the varus-valgus and internal-external laxity behavior of the knee, and we suggest that 10 Nm and 5 Nm moments are appropriate for these, respectively. It may also be helpful to use a less complex loading system to study these rotations in isolation, perhaps using simple arrangements of weights and pulleys to apply the desired torques, and similar considerations apply regarding the freedom of coupled motions [2].

Techniques to evaluate cruciate ligament graft fixation

There are many different animal models that can be used to evaluate ACL graft fixation, but the most important information regarding ultimate failure strength characteristics is probably gained from human specimens. This is because the normal intact knee can be evaluated, and thus, comparisons can be made to a known standard. It is clear that human specimens are not always available, and therefore, animal models may be a necessary substitute. With respect to most ACL graft fixation studies, the mechanical integrity of soft tissue and bone is of primary concern. For example, adult bovine cortical bone is much harder than human bone, and it may artificially inflate the structural material properties of an ACL graft-bone fixation construct, although it has been shown that bovine cancellous bone has much closer characteristics to young adult human cancellous bone than elderly human bone [8]. Other options include the ovine and porcine models, and these should be considered in light of the bone density, the soft-tissue material properties that are available in these specific models, and the size constraints of the joint. During selection of a model, it is important to identify whether young animals have cartilaginous regions about the interfaces between tendon/ligament and bone and the epiphyses of the tibia, femur, and patella. These regions may become the 'weak links' or failure sites during materials testing and not be representative of the failure mechanisms that occur in humans.

There are at least two different approaches that can be used to evaluate the biomechanical behavior of cruciate ligament graft fixation. The first, and most common, is the uniaxial, single cycle load-to-failure test. The second approach includes cyclic loading of the joint. The advantage of the first approach is that an upper limit of the graft-fixation construct strength is defined. This is useful information with regard to the behavior of the graft during unexpected loading events such as that associated with the loss of balance or a fall. The advantage of the second approach is that it considers the cyclic behavior of the graft-fixation construct and allows one to determine how changes may occur immediately postoperatively.

Single cycle load-to-failure evaluation

Different approaches can be used to evaluate the single cycle load-to-failure characteristics of a graft-fixation construct. The first includes uniaxial tensile failure testing of the ACL or ACL graft. Specimens are mounted in a materials testing system and held with fixtures designed to load the ACL or ACL graft to failure [6] (Fig. 2). Woo and co-workers have demonstrated the importance of proper ACL alignment with applied tensile load during ACL failure testing [27]. With this approach, the loading fixture allows placement of the joint in full passive extension with alignment of the ACL (or ACL graft) collinear with the materials test system loading axis. The orientations of the bones with respect to the loading axis should be recorded, since both the angle of flexion and direction of loading affect the ACL failure strength.

The tibia and femur are clamped in the loading fixture without dissection of the major knee ligaments or menisci. This ensures that the relationship of the femur relative to the tibia is anatomically correct at the onset of failure loading. Full extension of the joint is chosen as a test position because with the knee in full extension, the fibers of the ACL are in their most parallel alignment, and under tension. This approach is thought to produce reproducible structural and material properties, rather than progressive tearing across the width of the ligament. Once the tibia and femur are clamped, all structures other than the ligament (graft) under study are dissected free, and load is applied along the long axis of the soft tissue by the materials testing system, while displacement between the tibia and femur are recorded.

For most test conditions, the loading rate should be standardized, and this may be in terms of increase of load

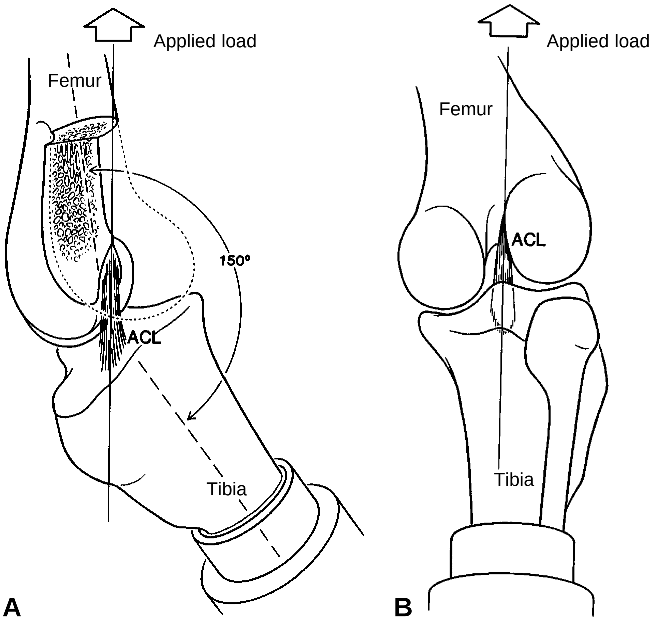

Fig. 2 A, B The failure testing fixture was designed to apply load along the axis of the canine anterior cruciate ligament (ACL), with all fiber bundles exposed to a similar load distribution. The measurements of the failure test include the load and displacement responses of the femur-ACL-tibia and the femur-graft-tibia complexes. Alignment is shown for the canine knee in full extension (30°) for the sagittal (**A**) and coronal (**B**) planes. For human specimens, this test would be performed in full extension (0°). (Reproduced with permission from [6])

(N/s), speed of distraction (mm/s), or strain rate, in which the speed of distraction bears a constant relationship to the initial (unloaded) ligament length. This is appropriate in view of the strain rate sensitivity of ligaments and their bony attachments. Since single cycle tests to failure relate to traumatic incidents, it is best to use a high rate of elongation. The anteromedial human ACL is approximately 33 mm long, and so a strain rate of 100%/s will require an actuator speed above 2 m/min. A slower speed can allow failure by bone avulsion, at a lower load [14]. The load and displacement data can be analyzed to determine the following characteristics of the soft-tissue-fixation construct: ultimate failure load, linear stiffness, displacement at failure, and the energy absorbed at failure. The ultimate failure stress and the failure strain data can be determined if the cross-sectional area of the soft-tissue structures are evaluated prior to failure testing. This can be accomplished with techniques such as direct contact measurement [21, 24], casting methods [16], or non-contact optical methods [26].

Many studies assume that crosshead or actuator movement represents ligament elongation, but there are also deflections under load in the mountings and the bones. This may sometimes be neglected in a test-retest protocol on the same knee, for example – but a more exact analysis of the ligament might require attachment of a displacement transducer or alignment of an optical tracking system to the ligament attachment areas.

Another approach to evaluating the single cycle load-to-failure characteristics is to apply an anteriorly directed load to the tibia using the fixture previously described to evaluate A-P laxity of the knee [19]. If this is done at 20° of flexion, this approach re-creates the Lachman exam, and therefore the results of this test are clinically relevant. If the failure test includes the ACL (graft) construct and contact between the tibial and femoral articular surfaces, then the results of this test may be difficult to interpret in terms of the standard structural and material properties of the graft-fixation construct.

Cyclic loading of the joint

The aforementioned investigation of the single cycle load-to-failure properties of ACL grafts at the time of implantation is important. In addition, attention should be paid to the mechanical behavior of the graft-fixation construct during healing where the environment is predominantly one of cyclic loading rather than single cycle failure loading. Most investigations of soft-tissue fixation to bone have focused on single cycle loading evaluations, or several cycles of pre-loading (pre-conditioning) followed by a single cycle failure evaluation. Another approach is to cyclically load the knee with a low peak force and observe the change in A-P displacement.

At present, the loads that are transmitted through an ACL graft during activities of daily living or rehabilitation are unclear. Graft loads are dependent upon what a subject chooses to do for exercise. The cruciate ligaments are the primary restraint to A-P displacement of the tibia relative to the femur [9]. Thus, we recommend applying A-P loads to the knee in a cyclic fashion using the techniques described previously to evaluate A-P laxity of the knee. One approach is to evaluate the A-P load-displacement response of the normal knee, section the ACL, perform the ACL reconstruction procedure, and repeat this testing protocol. We recommend loading between the limits of +150 N (anterior) and –150 N (posterior) and measuring the resulting displacement of the tibia relative to the femur for a selection of load cycles (this is commonly referred to as a load-defined or load-controlled test). We do not know how many load cycles are reasonable, but loading the knee through 1000 load cycles can evaluate the initial cyclic response of the graft without consideration of its biologic response. Broadly speaking, it will then be apparent if the situation is stable, or if repetitive slippage continues. These data could be analyzed to measure the relative increase in anterior displacement of the tibia relative to the femur as a function of the number of loading cycles. Data could then show the relative performances of graft fixations in terms of the number of load cycles needed to induce 1, 3, or 5 mm of excess anterior displacement of the tibia (relative to the normal, intact knee), for example. In addition, failure testing could be performed at the final load cycle to evaluate the structural properties of the graft-fixation construct. If tests are done in complete knees, it is essential that the orientations of graft fixation tunnels are specified, since angulation at the tunnel exit can have a large effect. An alternative approach is to apply the same cyclic load regime to a uniaxial specimen, with one bone fixed and the other end of the graft secured to the moving actuator [22]. An advantage of this approach is that it is controlled and allows slippage of a single fixation site to be monitored.

Conclusion

This paper arose from our discussions at a scientific workshop on ACL reconstruction sponsored by ESSKA. We found it very difficult to form opinions regarding the relative performances of a range of ACL graft fixation techniques because the authors had used different protocols, which were often not described fully. We realize that other workers in the field may have opinions which differ from ours, but we feel that the testing protocols described above are reasonable and appropriate, and we are putting them forward in the hope that their adoption by other research groups will allow comparison of results in the future.

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